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Basement Membrane Laminin and Type IV Collagen in Various Benign and Malignant Adnexal Tumors of the Skin: An Immunohistochemical Study

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Thirty benign and seven malignant adnexal tumors of the skin and one lymph node metastasis were stained for laminin and type IV collagen with rabbit antibodies against the human basement membrane (BM) proteins using the immunoperoxidase technique. Fifteen of the benign sweat gland, sebaceous gland, and hair follicle tumors showed a continuous and distinct BM around the tumor aggregates. The cylindromas and eccrine spiradenomas seemed to produce excessive amounts of BM material, part of which was seen as amorphic patches within the tumor cell clusters, whereas the trichofolliculomas, trichoepitheliomas, and pilomatrixomas showed an absence of BM from many areas. In syringomas, in addition to the tubular structures surrounded by a continuous BM, undifferentiated cell nests containing granular BM material were present. They probably represent primitive structures obtaining during early development into tubules. The seven malignant tumors and the only metastasis studied here all contained small, narrow strips of BM material extracellularly between the infiltrating tumor clusters. Only in two cases was faint staining for laminin found within the cells. The pepsin pretreatment of the formalin-fixed, paraffin-embedded samples had most probably degraded the intracytoplasmic BM material in most cases. The BM defects were found to be associated with malignancy and low differentiation of the adnexal skin tumors, as reported previously for other tumor types, but a partial loss of BM was also associated with high differentiation in some benign adnexal tumors.

TABLE I. *Basement membranes in adnexal tumors of the skin*

Origin and diagnosis	Number of cases	Basement membranes (continuous/discontinuous)	Numbers of leukocytes
Benign tumors			
Sweat gland	14		
Apocrine hidrocystoma	1	1/–	–
Nodular hidradenoma	2	2/–	–
Papillary hidradenoma	2	2/–	–
Eccrine hidradenoma	2	2/–	–
Cylindroma	2	2/–	–
Syringoma	5	–/5	–
Hair follicle	14		
Trichofolliculoma	3	2/1	–
Trichoepithelioma	4	–/4	–
Trichilemmoma	2	2/–	–
Pilomatrixoma	5	–/5	+
Sebaceous gland	2		
Sebaceous adenoma	2	2/–	–
Malignant tumors			
Sweat gland	5		
Hidradenocarcinoma	3	–/3	++
Apocrine hidradenocarcinoma	1	–/1	++
Nodular hidradenocarcinoma	1	–/1	+
Hair follicle	1		
Malignant pilomatrixoma	1	–/1	++
Sebaceous gland	1		
Sebaceous carcinoma	1	–/1	+
Metastasis			
Lymph node metastasis of sweat gland carcinoma	1	–/1	

The role of the basement membrane (BM) in tumor differentiation and growth has been a subject of intensive research lately. Chemically, BMs consist of both collagenous and noncollagenous glycoproteins (for reviews, see [1,2]). The scaffold of the lamina densa layers is formed by type IV collagen, which is organized in a netlike fashion [3]. The noncollagenous BM constituents comprise laminin (a glycoprotein of very high molecular weight), entactin, a heparan sulfate containing proteoglycan, and, at least in some BMs, fibronectin [1,2]. These components are probably mainly located in the lamina lucida layer although all the BM constituents have also been reported to be integrated into a common structure [4]. In addition to these constituents common to all BMs, some BM structures of limited tissue distribution are known, e.g., the bullous pemphigoid antigen, which is found in the BMs of stratified squamous epithelia [5].

Many benign epithelial tumors have an intact BM that can be visualized with antibodies to type IV collagen and laminin, whereas the BM in their malignant, invasive counterparts is either thin and discontinuous [6] or completely absent [6,7]. The tumor most systematically studied so far is breast carcinoma. No extracellular BM structures can be seen in its metastases [6,8], whereas intracytoplasmic staining both for laminin [6,8] and for type IV collagen [9] has been found. Some benign tumors of mesenchymal origin also produce laminin, and demonstration of this protein may be helpful in distinguishing among various types of soft tissue tumors [10].

Several studies have described the presence of a BM around basal cell carcinomas of the skin [11–15]. This BM is continuous and distinct in nonfibrosing tumors, but indistinct or discontinuous in fibrosing basocellular carcinomas [15]. There also seems to be a selective lack of the bullous pemphigoid antigen in the BM around basal cell carcinomas that distinguishes it from other types of skin tumor [11]. In contrast to

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Abbreviations:
BM: basement membrane

the basocellular carcinomas, there is only limited information available about the BM in adnexal tumors of the skin. Various benign adnexal tumors are quite common findings in dermatopathology, whereas their malignant counterparts are rare. It is not always easy, however, to tell which adnexal structure the tumor is differentiated toward or which structure it originates from.

We have now investigated 37 adnexal tumors with various degrees of differentiation, 30 benign, 7 malignant, with 1 lymph node metastasis using antibodies to human type IV collagen and laminin and have attempted to find correlations between the immunohistologic findings and the tumor type or the changes that take place in malignancy.

MATERIALS AND METHODS

Biopsy samples from 30 benign and 7 malignant adnexal tumors, with 1 lymph node metastasis, were selected from the files of the Department of Pathology, University of Oulu, and the Pathology Laboratory of the Cancer Foundation of Oulu, fixed in 10% formalin and embedded in paraffin. The sections, 5 μm in thickness were stained with hematoxylin and eosin, and the tumors classified into sweat gland, sebaceous gland, and hair follicle types (Table I). The number of leukocytes within and around the tumors was estimated on a scale of negligible (−), moderate (+), or abundant (++).

The 7-S collagen domain of type IV collagen was purified from human kidney [16] and the fragment P1 of laminin from human placenta [17], as described previously, and antisera to these proteins were raised in rabbits. The antibodies were purified by immunoabsorption on the relevant antigen coupled to Sepharose 4B. The laminin P1 antibodies were cross-absorbed with 7-S collagen and vice versa. There was no cross-reaction between the two antibodies in the radioimmunoassay.

FIG 1. Immunoperoxidase staining for the laminin fragment P1 and (*inset*) for the 7-S domain of type IV collagen in a cylindroma. The basement membranes around the tumor clusters, beneath the epidermis, and around the dermal capillaries are continuous and distinct. Abundant amorphic BM material is seen within some tumor clusters (*arrows and inset*) (× 44; *inset* × 90).

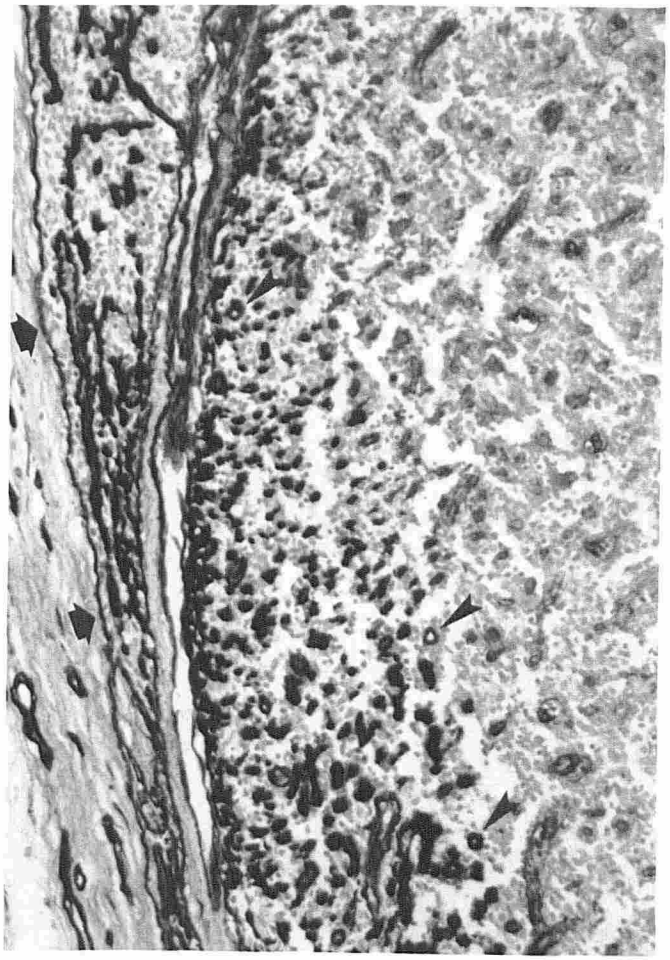

FIG 2. Immunoperoxidase staining for the 7-S domain of type 1 collagen in an eccrine spiradenoma. The tumor clusters are surrounded by a continuous BM (*arrows*). Amorphic BM material and capillaries (*arrowheads*) are seen within them (× 107).

The sections for immunohistochemical staining were deparaffinized and treated with 0.4% pepsin (Sigma Chemical Co., St. Louis, Missouri) to enhance the availability of the antigenic determinants [18,19]. The sections were exposed to a 0.1% solution of hydrogen peroxide in absolute methanol to inactivate the endogenous peroxidases, and then stained with anti-laminin P1 (20–40 μg/ml) or anti-7-S collagen (20–100 μg/ml) using the peroxidase-antiperoxidase procedure [20]. Normal rabbit serum and phosphate-buffered saline were used instead of the primary antibody for control stainings.

The BMs were analyzed and classified as follows: (1) continuous, when the BM was seen to envelop every tumor cell aggregate in a distinct, continuous line without disruptions, and (2) discontinuous, when the BM around some of the tumor cell aggregates was disrupted or not seen at all.

RESULTS

All the 37 samples of adnexal tumors of the skin were positive for both the laminin P1 and the 7-S collagen antigen, but showed variations in the integrity of the BM around the tumor cell aggregates. The detailed results are presented in Table I. The staining of the extracellular BMs for both antigens was identical in each case. The normal epidermal and capillary BMs were stained for laminin P1 and 7-S collagen in every sample, and the control stainings with normal rabbit serum and phosphate-buffered saline were all negative.

Fifteen of the 30 benign tumors showed a distinct continuous

BM around the tumor cell clusters. These included all the cases of apocrine hidrocystoma, nodular and papillary hidradenoma, eccrine spiradenoma, cylindroma, trichilemmoma, and sebaceous adenoma (Table I). The 2 cylindromas showed an unusually thick, homogeneously stained BM around the tumor cell nests and granular or amorphic BM material at the periphery or within the tumor clusters (Fig 1). This material stained equally with anti-7-S collagen and anti-laminin P1 antibodies. No intracytoplasmic staining was seen with certainty. The 2 eccrine spiradenomas contained not only a continuous BM around the large tumor cell islands and prominent capillary BMs, but also accumulations of laminin and type IV collagen material within the tumor cell aggregates, apparently extracellularly (Fig 2).

Fifteen benign tumors showed irregularities and disruptions in their BMs. The 5 syringomas contained both well-organized tubular structures surrounded by a distinct BM and unorganized cell nests which had indistinct, granular BM material around them (Fig 3). No intracytoplasmic staining was seen. Among the benign hair follicle tumors, 1 out of 3 trichofolliculomas, the 4 trichoepitheliomas, and the 5 pilomatrixomas were lacking in BM over some but not all areas (Fig 4). The pilomatrixomas were surrounded by a moderate number of leukocytes while all the other benign adnexal tumors were devoid of these (Table I).

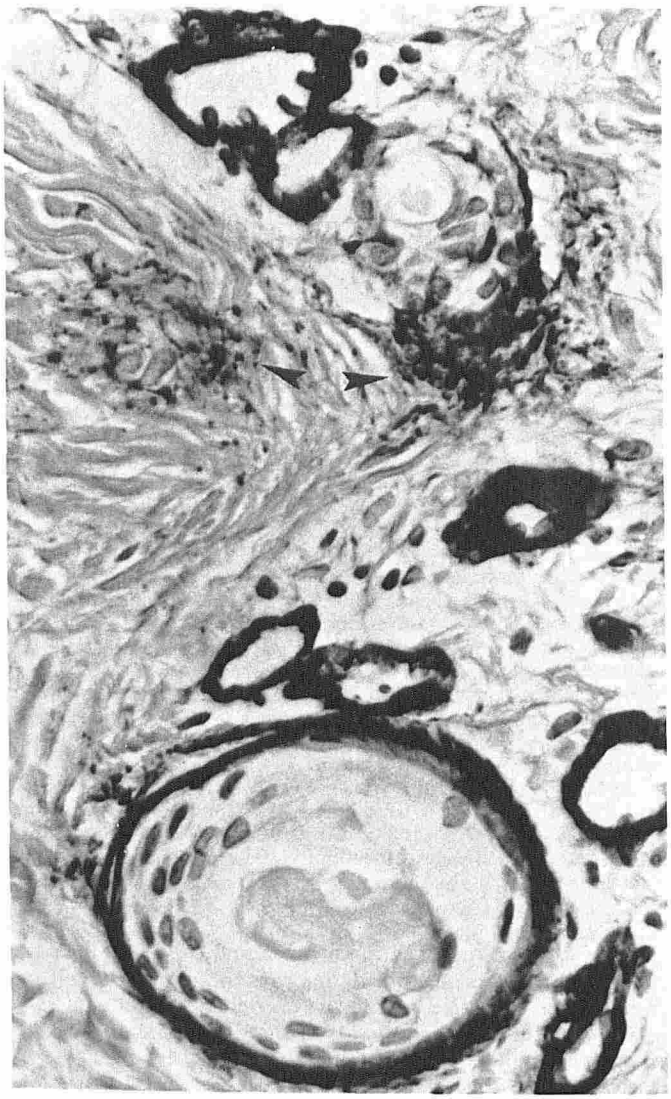

FIG 3. Immunoperoxidase staining for the 7-S domain of type IV collagen in a syringoma. The BMs around the dermal capillaries and neoplastic tubular structures are continuous, but only discontinuous, granular BM material (*arrowheads*) is seen around the unorganized, solid tumor cell nests (× 250).

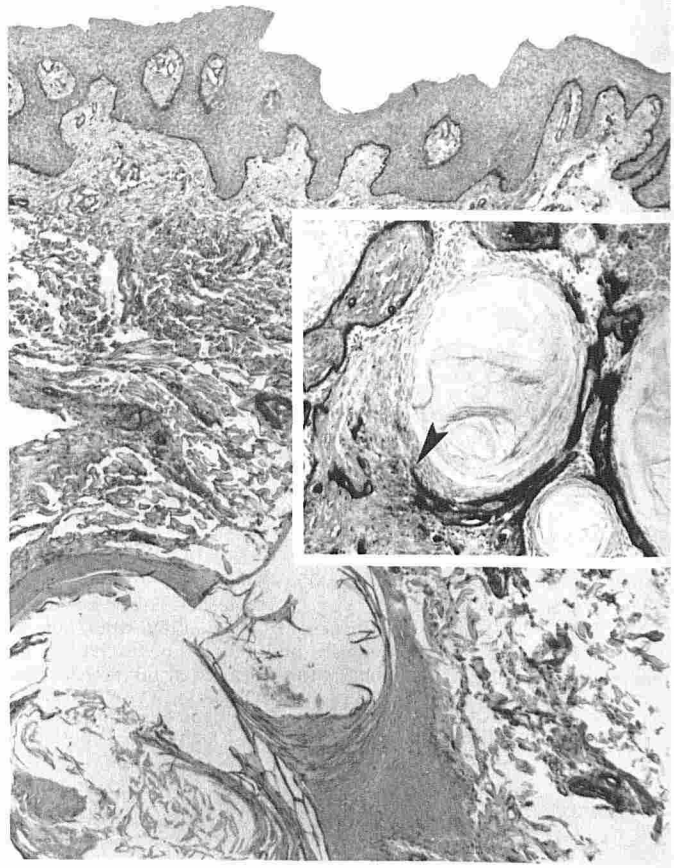

FIG 4. Immunoperoxidase staining for the 7-S domain of type IV collagen in a trichoepithelioma. The strongly keratinizing tumor epithelium has no BM, whereas that beneath the epidermis is seen clearly. The *inset* shows a disruption of the BM (*arrowhead*) around a keratinizing cyst in another trichoepithelioma (× 44; *inset* × 44).

The BM was discontinuous or lacking from wide areas in all of the 7 malignant adnexal tumors, 5 sweat gland, 1 sebaceous gland, and 1 hair follicle carcinoma, and in the only lymph node metastasis of a sweat gland carcinoma, but it was not totally absent from any tumor (Figs 5–7). Staining for both antigens revealed at least short, narrow strips of extracellular, linear BM material in all the skin tumors and in the metastasis (Fig 6). The largest BM-negative areas were those showing stromal invasion of the tumor cells or abundant leukocyte infiltrates (Figs 5, 7). All the adnexal carcinomas contained leukocytes around tumor infiltrates, most of them in abundant quantities (Table I), but disruptions of the BM were also seen in areas devoid of inflammatory cells. Two cases, the lymph node metastasis (but not its primary tumor) and one sweat gland carcinoma, showed intracytoplasmic staining for laminin (Figs 5, 6). None of the tumors showed intracytoplasmic 7-S collagen.

DISCUSSION

Antibodies against human laminin and type IV collagen were used here to characterize the BMs of various adnexal tumors. The presence of a continuous linear BM around a tumor suggests its benign nature, and this usually also holds for benign adnexal tumors of the skin. Some tumors form an exception, however. Some cells of trichofolliculomas, trichoepitheliomas, and pilomatrixomas, which are hair follicle tumors, show a loss

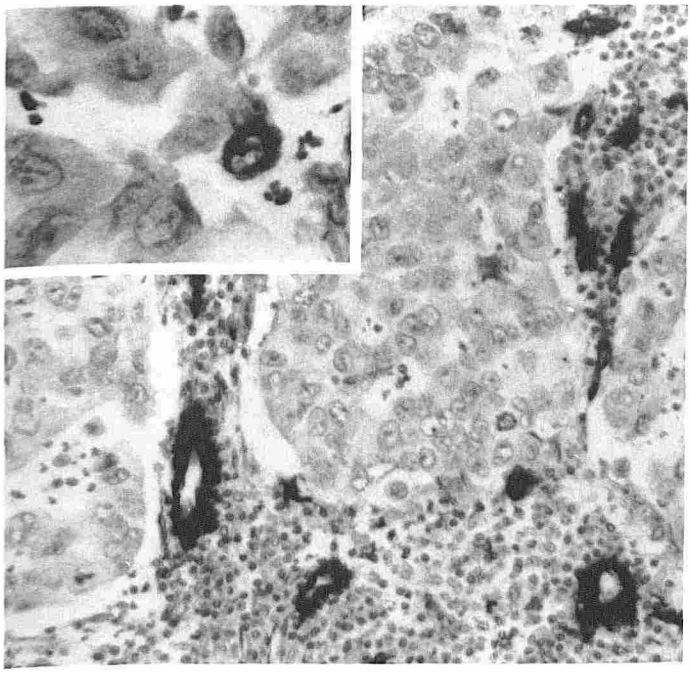

FIG 5. Immunoperoxidase staining for the 7-S domain of type IV collagen and (*inset*) for the laminin fragment P1 in a sweat gland carcinoma. The carcinoma cell clusters surrounded by large quantities of leukocyte infiltrates have no BM. One carcinoma cell shows intracytoplasmic staining for laminin (*inset*) (× 140; *inset* × 350).

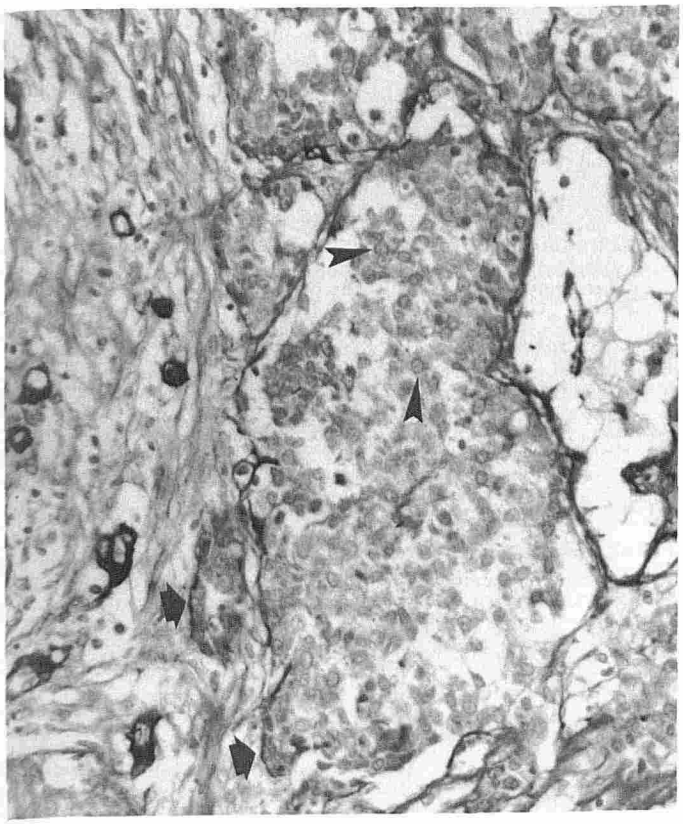

FIG 6. Immunoperoxidase staining for the laminin fragment P1 in the lymph node metastasis of a sweat gland carcinoma. Short, narrow BMs are seen at the periphery of some tumor clusters (*arrows*). Some tumor cells show intracytoplasmic staining (*arrowheads*). The capillaries are strongly stained (× 140).

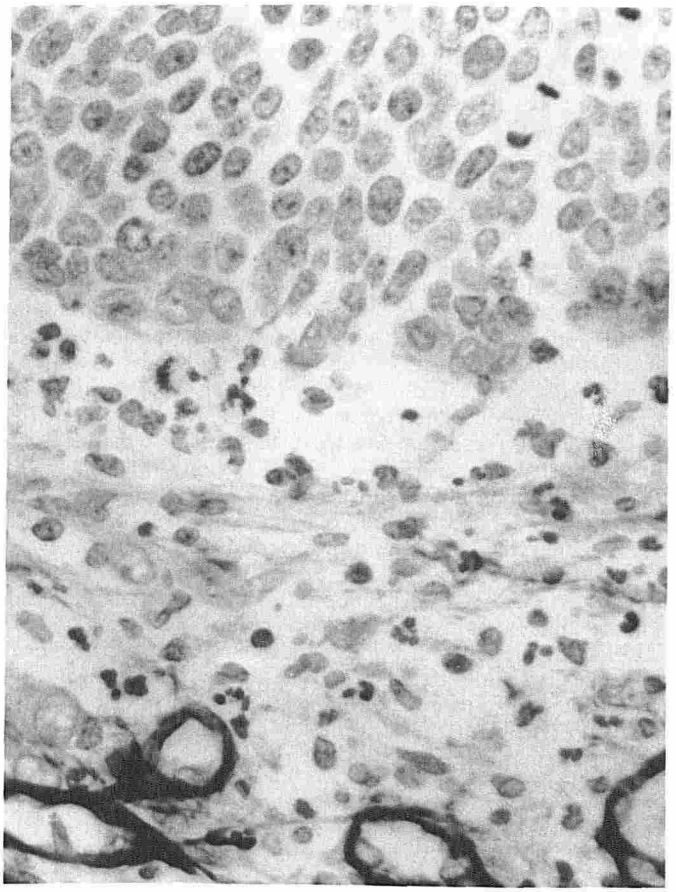

FIG 7. Immunoperoxidase staining for the 7-S domain of type IV collagen in a malignant pilomatrixoma. No BM is seen around the tumor epithelium surrounded by leukocytes. The dermal capillaries are strongly stained (× 350).

of the ability to produce BM material. Thus the partial lack of BMs in an epithelial tumor does not necessarily indicate a malignant potential, but differentiation with a loss of the capacity for BM production or maintenance.

Cylindroma cells, and to a lesser extent also spiradenoma cells, are able to produce excessive amounts of BM material (Figs 1, 2). The presence of amorphic BM material of this kind in cylindromas is in agreement with a previous report [11]. Contrary to that report, however, we did not find any difference in the distribution of laminin and type IV collagen. This slight disagreement could be due to the fact that our antibodies were directed against certain domains of human BM proteins, whereas in the previous paper antibodies against murine antigens had been used which have a partial immunologic cross-reaction with human antigens. Although this cross-reaction is sufficient for immunohistochemical purposes in most cases, there is some evidence of a reduced capability for antibodies against murine antigens to stain corresponding human antigens [21]. Also, the proteolytic pretreatment used by us could probably disclose some antigenic determinants that would otherwise be obscured.

The well-organized tubular structures in the syringomas had a normal-appearing, continuous BM around them, while the unorganized cell nests were unevenly surrounded by granular BM material (Fig 3). It is possible that these nests represent primitive structures in the early stages of organization into tubules. This situation could be analogous to that in developing kidney tissue, where the BMs around fully developed epithelial structures are preceded by granular material reacting with type IV collagen and laminin [22].

The absence of a continuous BM in the malignant adnexal tumors is in agreement with the findings concerning other types of epithelial malignancies [6–8,23]. A malignant epithelial cell apparently has a reduced capability to produce a BM, along with the fact that BM-degrading enzymes may be involved [24–26]. In any case, the inflammatory cells that are often present in malignant tumors are known to degrade BM [27–29]. The only metastasis studied contained extracellular laminin and type IV collagen and showed faint intracytoplasmic staining for laminin. This agrees with previous reports that metastatic epithelial cells are capable of producing BM material [6,8,9]. The absence of laminin staining inside the cells in many malignant tumors studied may be due to the proteolytic pretreatment of the samples, which has been shown to degrade intracytoplasmic laminin [6]. Preparation of the formalin-fixed, paraffin-embedded tissues used in this study nevertheless included pepsin digestion as a necessary step for demonstrating extracellular BM materials.

Laminin and type IV collagen immunostainings do not seem to be suitable diagnostic aids for discriminating between the various directions of adnexal differentiation, since both syringomas, being sweat gland tumors in origin, and trichofolliculomas, trichoepitheliomas, and pilomatrixomas which are hair follicle tumors, showed BM discontinuities. Similarly the BM stainings do not yield any simple results regarding the problem of the degree of differentiation. BM defects may be associated with pronounced cellular differentiation, as in trichofolliculomas and trichoepitheliomas, or low differentiation, as in syringomas and malignant tumors.

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